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P-332 Decoding autophagy dynamics in adenomyosis: insights of a murine model

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Study question: This study aimed to highlight the role of autophagy in the pathogenesis of adenomyosis using a murine model of medically induced adenomyosis.

Summary answer: Autophagy seems to play a role in the early stages of the disease and is likely not the primary mechanism driving the progression of adenomyosis.

What is known already: Autophagy is an intracellular mechanism involved in the recycling of aging organelles and non-functional proteins. Initially described as a phenomenon activated under stress or fasting conditions (non-selective autophagy), it is now accepted that a basal level of autophagy is essential for several cellular functions to maintain cellular homeostasis (selective autophagy). Autophagy is essential for cell survival and tissue growth, and an alteration of the autophagic pathway has been described in endometrial pathologies such as endometrial hyperplasia and carcinoma, infertility, and endometriosis. The contribution of autophagy to adenomyosis is still unclear due to conflicting findings in the existing literature.

Study design, size, duration: This experimental study was conducted from October 2022 until January 2024. A total of 170 mice pups were treated (90 treated with tamoxifen – 80 with vehicle only).

Participants/materials, setting, methods: Female neonatal CD1 mice received oral doses of 2.7µmol/kg tamoxifen from days 2 to 5 after birth. Control mice received only the vehicle. After synchronizing their estrus cycle, the mice were euthanized at 1 month or 3 months old. The diagnostic and grading of adenomyosis were performed by histological examination (hematoxylin-eosin and immuno-fluorescence). RT-PCR and Western Blot were used to study the modulation of autophagy pathways between the uterus of the two groups.

Main results and the role of chance: Histological analysis of 3-month-old tamoxifen-treated group showed that 97% of the mice developed the disease. The grading method was defined by assessing the depth of lesions. After sensitivity enhancement through immunofluorescence staining (alpha-SMA, EpCAM and DAPI), grade 3 adenomyosis was diagnosed in up to 91% of our samples (vs 84% when analyzed by H&E only). Neonatal treatment of CD1 mice with tamoxifen reliably produces severe adenomyotic lesions. Immunofluorescence enhances sensitivity in histological examinations of myometrial lesions, combining alpha-SMA staining for smooth muscle detection with EpCAM for identifying epithelial cells.

RTqPCR analysis of lc3b, akt, bcl2, bclxl, ulk2, ulk1, cxcr4, tnfs10, dapk1, igf1, atg9, pik3c3, and bid showed an up-regulation of autophagy in one-month-old adenomyosis mice. This was also indicated by a decrease in akt and bcl2, which are inhibitors of the pathway, along with an increase in lc3b, a marker for phagophore formation. However, the conflicting decrease in ulk2, a component of the induction complex, raises questions. Importantly, these changes in autophagy are not observed in 3-month-old mice, suggesting that autophagy may play a role in the early stages of disease development. There were no statistical differences in Protein expression of pakt/akt, lc3b, and bcl2 between the groups.

Limitations, reasons for caution: The analysis used protein or mRNA extracted from the whole uterus. Variations in results may arise if the endometrial and myometrial components are analyzed separately. Techniques like laser capture microdissection could prove valuable in this context. Continued research focusing on investigating the role of apoptosis in the study is essential.

Wider implications of the findings: Given the frequent association of adenomyosis with endometriosis and/or uterine fibroids, using an experimental model offers the advantage of eliminating bias from associated comorbidities. This approach presents a unique opportunity to unravel the molecular basis of adenomyosis, while enabling a translational analysis when juxtaposed with clinical studies.

Trial registration number: not applicable